

Pulsed-Field Gel Electrophoresis PFGE: What does it do for you?

ERRT Conference

Laura Iwig

July 31, 2012



Pulsed-Field Gel Electrophoresis: Hospital to Division of Laboratory Services

- Patient seeks medical attention and specimen collection
- Testing at hospital or reference lab
- Positive for Salmonella, Shigella, E. coli, Campylobacter
- Isolate is sent to KY Division of Laboratory Services for serotyping and PFGE



At DLS

- Bacteriology lab will grow isolated colony:
 - If not isolated PFGE will not yield viable results
- PFGE lab after BaCT confirmation



<http://blogs.cdc.gov/publichealthmatters/2009/07/not-a-typical-spring-weekend-seven-salmonella-investigations/>

Bacterial Suspension



Mix with Agarose

Plug Mold



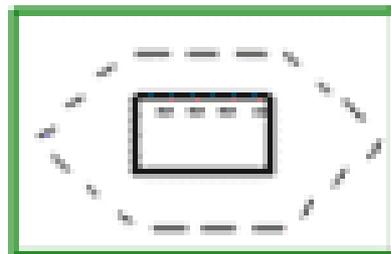
Chemical Lysis and Washing



DNA in Plugs



Restriction Enzyme



Electrophoresis (PFGE)



Documentation (capture gel image)

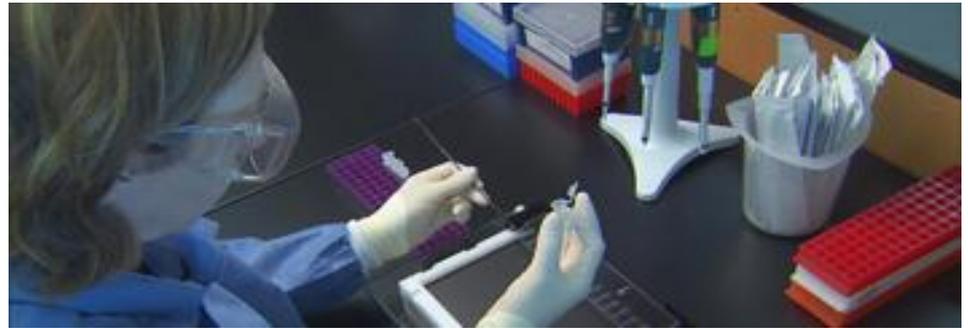


Data Analysis

<http://www.cdc.gov>

Steps of PFGE

- Isolate Colony
- Prepare Buffers and Agarose
- Make Plugs
- Lysis of cells
- Wash Plugs
- Prepare and load gel



<http://www.cdc.gov/features/ds/PulseNetFoodborneIllness/>

PFGE Steps Continued

- Run Electrophoresis (18-19 hours)
- Imaging
- Analysis of image
- Send to CDC for pattern allocation



<http://www.pulsenetinternational.org/networks/Pages/newzealand.aspx>

Time Line

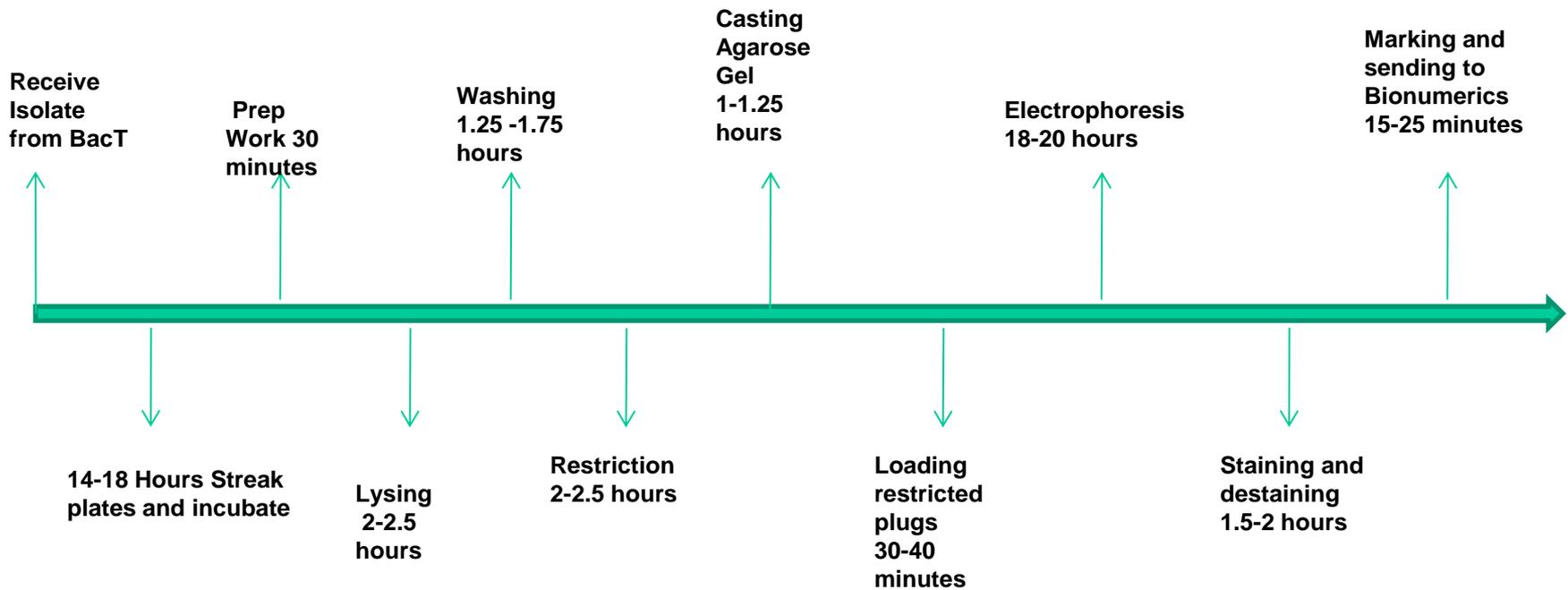
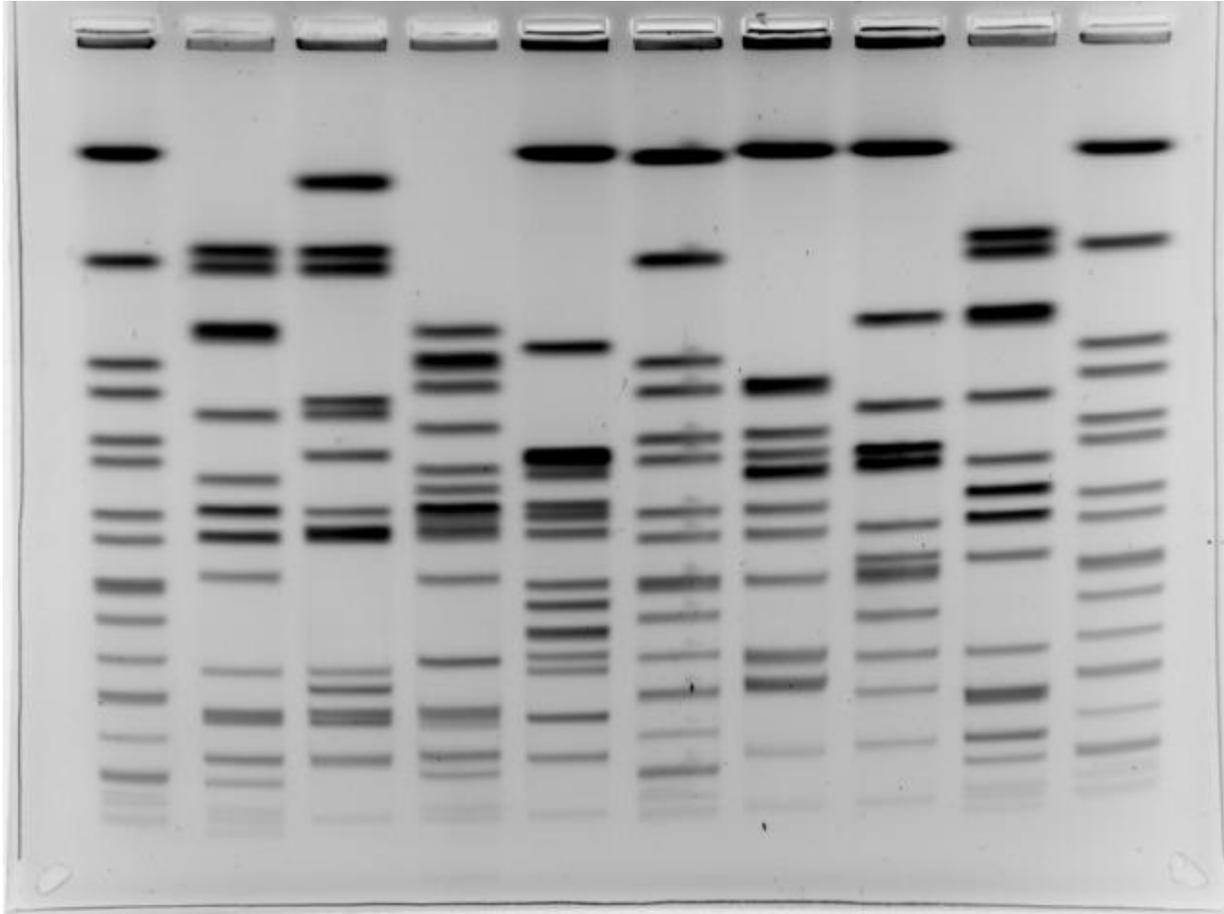


Image for Analysis



Communication with CDC: Assigning Pattern Names

- Marked images are sent to CDC electronically
- Patterns retrieved by connecting to CDC server
- Can take several days for CDC to assign pattern to isolate

Bionumerics

BioNumerics

File Edit Database Subsets Experiments Comparison Identification PulseNet Scripts Help Window

Complete view

Key	Index	LabID	Serotype	OGroup	PFGE-XbaI-p...	PFGE-BlnI-p...	Outbreak	Source
12-22205241-0146	4143	KY	I 4,[5],12:iii-	B (O 4)	JPXX01.1314		1108VTJJPX-1	KY
12-22233229-0150	4144	KY	Enteritidis	D1 (O 9)	JEGX01.0034			KY
12-22233233-0151	4145	KY	Enteritidis	D1 (O 9)	JEGX01.0004			KY
12-22254125-0133	4146	KY	Muenchen	C2 (O 8)	JJ6X01.2799			KY
12-22261617-0148	4147	KY	Serotype pending		JAXX01.0038			KY
12-22261861-0147	4148	KY	Serotype pending					KY
12-22296929-0156	4149	KY	Enteritidis	D1 (O 9)	JEGX01.0005			KY
12-22365846-0162	4150	KY	Poona	6 (O 13)	JL6X01.0104		1203MLJL6-1	KY
12-22395262-0160	4151	KY	I 4,[5],12:iii-	B (O 4)	JPXX01.2583		1202MLJJPX-1	KY
12-22396087-0161	4152	KY	Serotype pending		TdLX01.0038			KY
12-22413494-0169	4153	KY	Serotype pending		JEGX01.0034			KY
12-22428711-0172	4154	KY	I 4,[5],12:iii-	B (O 4)	JPXX01.0958			KY
12-22487164-0185	4155	KY	Enteritidis	D1 (O 9)	JEGX01.0004			KY
12-22518020-0173	4156	KY	Enteritidis	D1 (O 9)	JEGX01.0002			KY
12-22537904-0177	4157	KY	Typhimurium	B (O 4)	JPXX01.0150			KY
12-22568487-0214	4158	KY	Kiambu				TN	
12-22598937-0186	4159	KY	Newport	C2 (O 8)	JJPX01.1466			KY
12-22606760-0178	4160	KY	Newport	C2 (O 8)	JJPX01.3914			KY
12-22632994-0203	4161	KY	Serotype pending					KY
12-22633071-0202	4162	KY	Mississippi	6 (O 13)	JJPX01.0015			KY
12-22654207-0193	4163	KY	Serotype pending		JL6X01.0578			KY
12-22695654-0200	4164	KY	Enteritidis	D1 (O 9)	JEGX01.0034			KY
12-22740358-0216	4165	KY	Typhimurium	B (O 4)	JPXX01.1017			KY
12-22869053-0210	4166	KY	I 4,[5],12:iii-	B (O 4)	JPXX01.1637			KY
12-22875036-0206	4167	KY	Serotype pending		J66X01.0095		1203MIJ66-1	KY
12-22938586-0215	4168	KY	Isolate to CDC					KY
12-22977495-0222	4169	KY	Oranienburg	C1 (O 7)	JJXX01.0028			KY
12-23101564-0230	4170	KY	Typhimurium	B (O 4)	JPXX01.2379			KY
12-23247976-0238	4171	KY	Serotype pending		JPXX01.1637			KY
12-23293471-0239	4172	KY	Newport	C2 (O 8)	JJPX01.1132			KY
12-23412600-0245	4173	KY	Enteritidis	D1 (O 9)	JEGX01.0021			KY
12-23434828-0246	4174	KY	Enteritidis	D1 (O 9)	JEGX01.0004			KY
12-23436673-0251	4175	KY	Newport	C2 (O 8)	JJPX01.0413		1204OHJJJP-1	KY
12-23482740-0252	4176	KY	Hartford	C1 (O 7)				KY
12-23527890-0253	4177	KY	Newport	C2 (O 8)	JJPX01.0413		1204OHJJJP-1	KY
12-23594219-0259	4178	KY	Altana	C2 (O 8)				KY
12-23629067-0272	4179	KY	Enteritidis	D1 (O 9)	JEGX01.0034			KY
12-23632525-0270	4180	KY	Montevideo	C1 (O 7)	JIXX01.0998		1204MOJIX-1	KY
12-23637908-0271	4181	KY	Serotype pending		JAXX01.0001			KY
12-23670203-0274	4182	KY	Enteritidis	D1 (O 9)	JEGX01.0004			KY
12-23670496-0275	4183	KY	Newport	C2 (O 8)	JJPX01.0413		1204OHJJJP-1	KY
12-23794989-0279	4184	KY	Heidelberg	B (O 4)	JF6X01.0581			KY

Experiments

Name
1 PFGE-XbaI
2 PFGE-BlnI
3 PFGE-SpeI
4 XbaI+BlnI
5 AvrII
6 Salmonella XbaI
7 GelCompar
8 NH03149
9 EC-XbaI
10 Bpertussis
11 ...

Files

Name	Cr
N 11001	
12050	20
AB04051.tif(1)	20
AB04059	20
N AB04088	
AZ04055	20
CDC03Sal-PTa	20
CDC04SAL-PT	20
CDC05SA-PT	20
N CDC06SA-PT	

Comparisons

Name	Cr
KY Salmonella Uni...	2012-

All levels

Database: Salmonella1 4507 entries 20 experiments C:\Program Files\BioNumerics\data\Salmonella1

Start | Inbox - Mailbox - Grooms... | BioNumerics

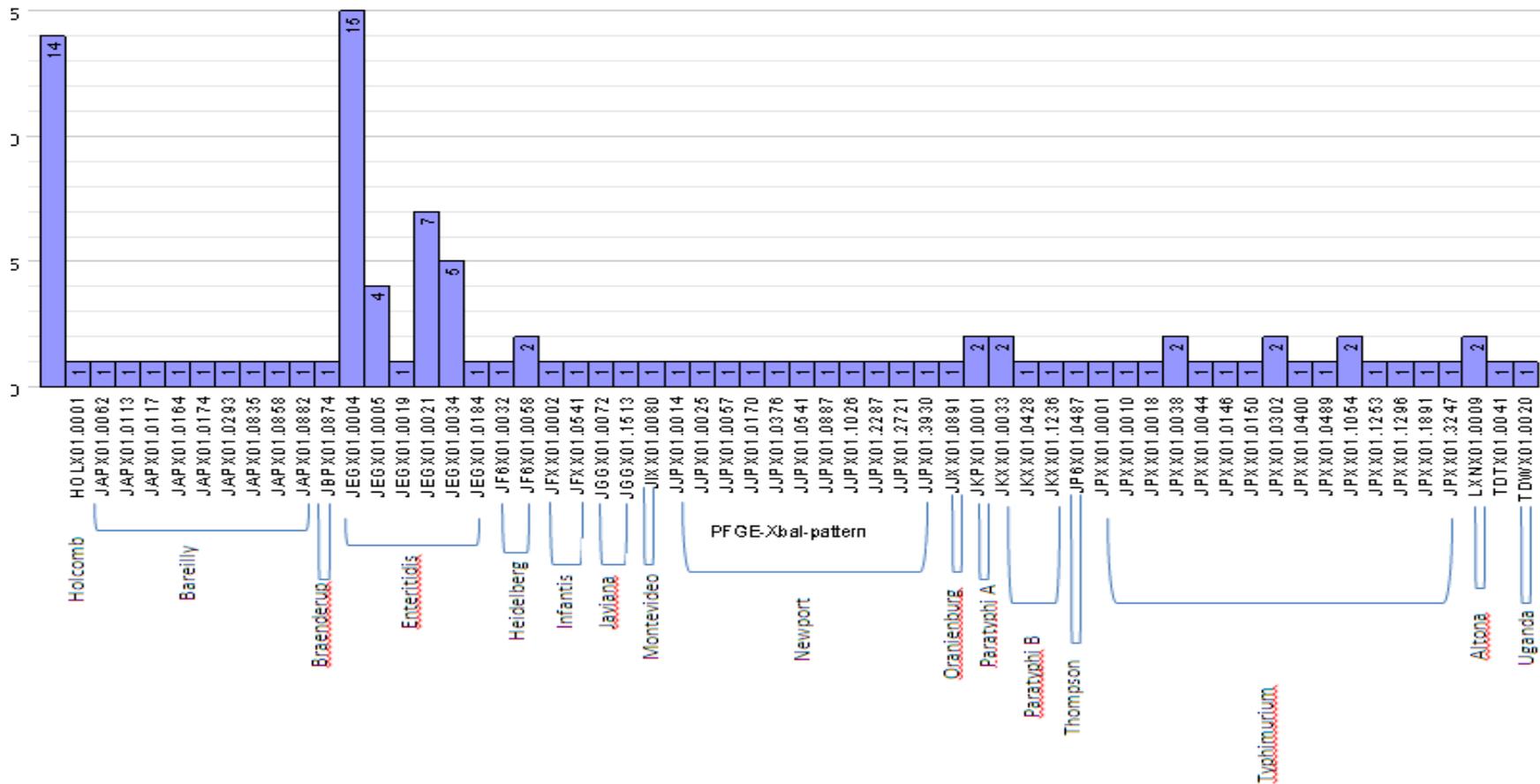
12:03 PM



Surveillance Activities

- Weekly surveillance: 60 day counts in BioNumerics
- Constant communication with Reportable Diseases Section
- Monthly meetings with Reportable Diseases

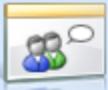
60 Day Salmonella Count: July, 2012



Reporting a Cluster

- Salmonella: 3 or more isolates with matching PFGE patterns or 2x baseline for more common PFGE patterns
- E. Coli: report 2 or more isolates with matching PFGE pattern to PulseNet
- Shigella Sonnei: more than 3 isolates with matching PFGE patterns with additional investigation
- All other Shigella Serotypes: report all clusters with 2 more matching PFGE patterns

SharePoint Posting



Salmonella (JEGX01.0021)_KY_Enteritidis

View All Site Content

Documents

- Library of PulseNet Documents
- Library of OutbreakNet Documents

Announcements

- PulseNet Announcements
- OutbreakNet Announcements

Discussions

- 2012 Update Meeting
- E. coli
- Listeria
- Salmonella
- Campylobacter
- Botulism
- Shigella
- Vibrio
- Other Organisms
- Unknown Etiology Illness
- GI Viruses/Norovirus
- Regulatory Issues/Public Policy
- Troubleshooting BioNumerics

Discussion board for Salmonella

Actions ▾

ID Post

Created

Created By

Started: 7/16/2012 3:19 PM

1650 (JEGX01.0021)_KY_Enteritidis

7/16/2012 3:19 PM

McGlinchey

KY appears to have a cluster of 7 *S. enteritidis* that match by XbaI. Key ID are as follows:

Key Id	County	Age	Sex	DOC
12-24035897-0310	Nelson	71	F	4/12/2012
12-25177232-0453	Jefferson	70	M	5/13/2012
12-25474154-0522	Casey	52	F	6/5/2012
12-25575475-0533	Boone	19	F	6/6/2012
12-25576212-0534	Boone	34	F	6/8/2012
12-25588219-0544	Fayette	31	F	6/5/2012
12-25692918-0556	Grant	7	M	6/11/2012

Bundle file is attached.

Epi has been informed.

Epi Contact:

Laura Iwig

laura.iwig@ky.gov

SharePoint Response

<ul style="list-style-type: none">GI Viruses/NorovirusRegulatory Issues/Public PolicyTroubleshooting BioNumericsTroubleshooting LabSharePoint Comments <p>Subsites</p> <ul style="list-style-type: none">Non-Disclosure Agreement Subsite	<p>Laura Iwig laura.iwig@ky.gov</p>		
<p>Posted: 7/16/2012 3:24 PM</p>			
	<p>1651 Hello, These Enteritidis isolates posted by KY have been assigned pattern JEGX01.0021. This pattern has been seen 229 times in the past 60 days. Currently this is not an increase at the national level; therefore, a cluster code will not be assigned at this time. We will continue to monitor this pattern for future uploads. Please continue to update this posting with any additional information. Thank you, Beth McGlinchey</p>	<p>7/16/2012 3:24 PM</p>	<p>McGlinchey</p>

SharePoint Posting

Documents

Announcements

- PulseNet Announcements
- OutbreakNet Announcements

Discussions

- 2012 Update Meeting
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- Other Organisms
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- Troubleshooting BioNumerics
- Troubleshooting Lab
- SharePoint Comments

Subsites

- Non-Disclosure Agreement Subsite

1533 **1207KYJBP-1 (JBPX01.0874)_KY_Braenderup**

7/9/2012 10:26 AM

Im, S

Good Afternoon,

KY appears to have a cluster of S. Braenderup. This pattern is new to the KY database. Key ID and collection date are as follows:

Key ID	County	Age	Sex	DOC
12-25349231-0491	Boyle	22	F	05/31/2012
12-25527771-0527	Jefferson	5	M	06/03/2012
12-25535369-0557	Christian	23	M	06/04/2012
12-25639570-0548	Hardin	0-21 M	M	06/08/2012

The bundle file is attached.

Epi Contact:

Laura Iwig

Laura.iwig@ky.gov

502-564-4446 x 4454

SharePoint Posting

Posted: 7/9/2012 10:28 AM

1534 Hello Everyone,

Kentucky initially posted their original message on 6/28/2012 and the cluster was observed for a week before assigning a cluster code.

This cluster of *Salmonella* Braenderup has been given the cluster code 1207KYJBP-1. The PFGE XbaI Pattern associated with this cluster is JBPX01.0874, comprising 0%(0/6537) of *S. Braenderup* isolates in the database.

In the last 60 days, this pattern has been seen 8 times from the following states:

SourceStates: AK, KY (4), NC, VA, WV;
LabIDs: AK, KY (4), NC, VA, WV.

Please find attached a line list and bundle file.

The pattern associated with this cluster (JBPX01.0874) is a new pattern in the *Salmonella* database. Please continue to post updates and epi information.

Thank You,

Sung Im
PulseNet Database Administration Team
Phone: (404) 639-4558
PulseNet: (404) 639-4558

7/9/2012 10:28 AM

Im, Sung (CDC/OID/NCEZI)

Special Requests

- In outbreak situations, isolates can be prioritized
- PFGE process cannot be sped up (3-4 day process)

Thanks to:

- Joshua Tobias, PhD
- William Grooms



Questions?

